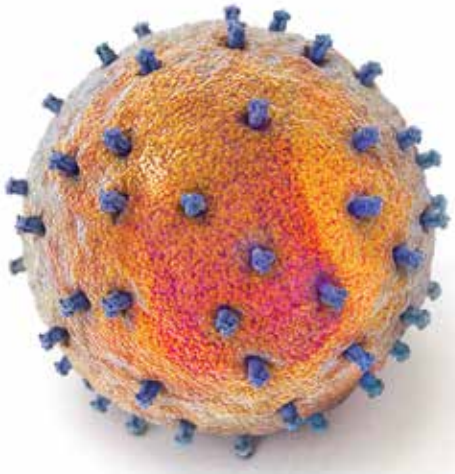
NOVEMBER 2013 | VOLUME 11 | NUMBER 6

vax

The Bulletin on AIDS Vaccine Research



[SPOTLIGHT]

Barcelona, in Essence

Our take on the key talks and takeaways from AIDS Vaccine 2013 *By Unmesh Kher*

Back in the mid-1980s, when scientists first tried to design AIDS vaccines, the task seemed pretty straightforward. They took a piece of HIV's envelope—a toadstool-like protein complex that traverses the outer membrane of the virus—injected it into folks and waited to see if the immune system would do the rest. The strategy itself was rational, and far from unprecedented: viral vaccines work mainly by inducing antibodies against such surface molecules, known as antigens. Yet the result, after quite a lot of waiting, was a serious letdown. And the blame lay not so much within the immune system (at least, not completely) as with the HIV antigens used.

HIV, it turns out, is far too variable and cunning to succumb to such old-school strategies. But over the past couple of decades, researchers have learned a lot more about how the virus befuddles the immune response, and how that capability might be disrupted. And if the proceedings at AIDS Vaccine 2013 in Barcelona in early October were any indication, they are beginning to figure out how to mine that knowledge to make potentially better antigens, deliver them more effectively and perhaps—someday, in the not too distant future—make reasonably effective HIV vaccines.

A useful trimer mimic?

The work that made the biggest splash in Barcelona was from a team of researchers led by Ian Wilson and Andrew Ward of The Scripps Research Institute in La Jolla, California; John Moore of Cornell University in New York; and Rogier Sanders of Cornell and the Academic Medical Center in Amsterdam. The team described in a series of talks how they had constructed a promising mimic of the external part of the envelope protein—also known as the trimer—and determined its structure using two distinct techniques. Most importantly, they revealed that the mimic appears to be nearly identical in structure to the external portion of the natural trimer, at least to the extent that this structure is known.

This matters because the envelope is the only target on HIV available to antibodies, and so of great significance to HIV vaccine design. It is, however, a highly mutable protein that has also evolved a number of other tricks to evade the antibody response. To make matters worse, it is terribly finicky and falls apart very quickly, confounding efforts to both probe its functional structure and to use it as a vaccine antigen, which is known as an immunogen. The trimer made by John Moore and colleagues, on the other hand, has been engineered to assemble appropriately and remain stable in solution, permitting its structural analysis.

The trimer mimic, named BG505-SOSIP.664, binds to pretty much all subsets of known broadly neutralizing antibodies (bNAbs), so named because they target the envelope protein in a manner that disables most circulating varieties of HIV in laboratory studies. This has two major implications. First, it is further evidence that the trimer mimic is virtually identical to its natural counterpart, since some bNAbs only bind to appropriately structured trimers. Second, it opens the door to using it as an immunogen that might teach the body how to make bNAbs and so thwart HIV (see box, page 3).

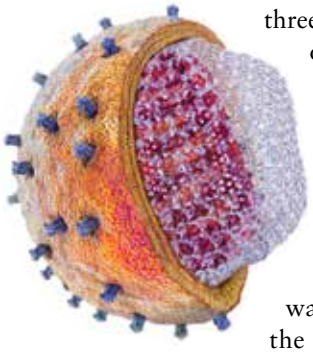
For now, though, the mimic remains a work in progress for such uses. Still, its creation and structural analysis was largely received as a major achievement both at the conference and after its publication in *Science*.

ALSO IN THIS ISSUE

Coaxing out the bNAbs

Yet simply making the right immunogen isn't likely to suffice, since bNAbs take several years to mature and any vaccine worth its salt will have to induce them far faster than that. Penny Moore of the National Institute of Communicable Diseases in South Africa described in what she called "excruciating detail" how a game of cat and mouse between HIV and antibodies in a single HIV-infected person fueled the evolution of bNAbs.

The talk was indeed detailed, but fascinating nonetheless. Moore's analysis showed how the antibody response in her one chosen subject came in three distinct waves. Each of the first two waves applied pressure on the virus, which mutated to evade the attack. But in doing so, it inadvertently exposed a weakness, which was then targeted by the succeeding wave of antibodies. In the end, three successive waves of antibodies generated a highly potent and broadly neutralizing antibody to HIV.



Moore's detailed description of the viral and antibody mutations that drove this game to its conclusion add to a growing body of data that might permit the precise design of antigens and vaccination regimens to hasten the maturation of bNAbs. Indeed, Barton Haynes of Duke University presented early data on his development of such regimens.

Interestingly, one additional bNAb-related talk that caused some excitement at the conference was not about the role of these antibodies in vaccine responses, but their uses in therapy and possibly as a tool for cure research (see *Global News*, this issue).

The Thai trial keeps on giving

Another pair of talks, however, reminded attendees that for all the fuss about bNAbs and their generation, the sole vaccine regimen to have provided any measure of protection from HIV induced not one detectable bNAb. A talk by Merlin Robb of the US Military HIV Research Program summed up what researchers have learned about how protection might have been induced in this trial, RV144, which was completed in 2009 in Thailand and showed a 31% efficacy overall after three and a half years (though protection appears to have been nearly twice that high in the first year following vaccination).

To recap: the analysis of samples collected in that trial revealed that certain classes of antibodies, known as IgGs, that target highly variable regions of the trimer were essential to the reduction in risk among vaccine recipients. Robb also said that more recent evidence suggests similar antibodies to a third variable region of the envelope might have played a role. These antibodies are not "neutralizing," which is to say they cannot block HIV entry into its target cell.

Previous research has separately confirmed these findings: mutations HIV had to obtain to escape protection correspond perfectly with the spot on the variable region that appears to be targeted by such antibodies. At the same time, the presence of IgA antibodies against the envelope correlated to increased risk for infection in vaccine recipients.

This makes sense because non-neutralizing antibodies work by directing other components of the immune response to destroy infected cells. IgGs, for example, bind immune cells known as natural killer cells and promote the destruction of infected cells by these "NK cells." IgAs do not induce this sort of antibody-dependent cellular cytotoxicity (ADCC). But what they can do, if they cluster around the envelope protein, is keep effective IgGs from doing their work.

Antibodies, sugars, and subtypes

Other studies led by the laboratories of Georgia Tomaras of the Duke Human Vaccine Institute and Galit Alter of the Ragon Institute in Boston, Massachusetts, suggest that a subclass of IgGs called IgG3s appear to have been essential to the protection afforded by the RV144 regimen. A comparative analysis reveals that another trial, VAX003, which failed to find any sign of protection, primarily elicited IgG1, 2, and 4, while only RV144 elicited higher levels of IgG3.

It also turns out that the IgG3s elicited by RV144 are more functional than those elicited by VAX003. That is, they induce ADCC better than the IgG3s elicited in VAX003, and greater functionality in this subclass of IgGs correlated with reduced risk in RV144. Most of the IgG3s induced by VAX003 recruit one or two types of responses from other players of the immune system. The IgG3s from RV144, meanwhile, recruit three or more such responses (with a mean 4.6 "effector functions" induced).

Alison Mahan described a fascinating series of analyses conducted on antibodies collected from the two trials in Alter's lab, where she is a graduate student. She and her colleagues found that the antibodies elicited by VAX003 tended to be modified by glycosylation—the addition of complex sugar chains—in a manner that resulted in drawing immune responses that promote inflammation. Those induced by RV144, on the other hand, were less inflammatory and more tuned to inducing immune responses that lead to the elimination of virally infected cells.

Mahan and her colleagues also compared samples from the Step trial, which tested a vector based on Ad5, and from a trial that tested an Ad26 vector along the same lines—measuring 67 different immunologic parameters. Intriguingly, responses from the Ad5 vector resembled those obtained in VAX003, while the Ad26 responses clus-

EDITOR
Unmesh Kher

SENIOR SCIENCE WRITER
Andreas von Bubnoff, PhD

SENIOR PRODUCTION MANAGER
Nicole Sender

FREE SUBSCRIPTIONS:

To obtain a FREE subscription to *VAX* by e-mail or change your subscription details, please go to www.vaxreport.org and click on the Subscribe link.

VAX is a bi-monthly bulletin from IAVI Report, the independent publication on AIDS vaccine research published by the International AIDS Vaccine Initiative (IAVI). It is currently available in English and Spanish as a downloadable PDF file or an e-mail bulletin.

The International AIDS Vaccine Initiative (IAVI) is a global not-for-profit organization whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. Founded in 1996, IAVI works with partners in 25 countries to research, design and develop AIDS vaccine candidates. In addition, IAVI conducts policy analyses and serves as an advocate for the AIDS vaccine field. For more information, see www.iavi.org.

tered with those detected in RV144. But perhaps the most interesting takeaway from this talk was the suggestion that different vaccination strategies can induce different degrees of inflammatory and functional immune responses through subtle changes in the kinds of antibodies induced.

Ad5 has now failed dismally in two large-scale efficacy trials, and will likely never again be touched as a vehicle for the delivery of HIV immunogens. Indeed, results from an ongoing analysis of Ad5 clinical trial data by Peter Gilbert of the Fred Hutchison Cancer Research Center suggests that the higher risk of HIV infection observed in vaccine recipients in the Step trial was real. But all agreement stops there. Even the question of whether that effect was linked to the vaccine candidate itself is being debated, and it remains unclear how the candidate or the regimen might have induced that effect.

Targeting infected cells

Effective HIV vaccines will in all likelihood also have to engage the T-cell response, which is essential to eliminating cells infected by HIV. Some viruses are, after all, likely to evade any antibody elicited by vaccination. Devising such vaccines has proven to be quite a challenge as well, since T-cell targets too are highly variable when obtained from HIV. So researchers have lately tried a few strategies to devise immunogens to focus T-cell responses on those parts of the virus that are not subject to much change.

One such strategy presents the immune system with an immunogen encoding just the conserved sequences of HIV proteins. Thomas Hanke of the Jenner Institute in Oxford presented data from the first clinical evaluation of vaccine candidates carrying such an immunogen, which encode conserved regions of the HIV envelope protein. His data showed both a breadth and a potency of cytotoxic T-cell responses that exceeded those observed previously with vaccines targeting the whole envelope protein.

The field has also witnessed exciting advances in the delivery of immunogens, especially the development of vectors that retain the ability to multiply following delivery—potentially eliciting more sustained and effective immune responses. Louis Picker of the Oregon Health & Science University gave a well-attended talk on the preclinical results his laboratory has obtained with a replicating vector derived from cytomegalovirus (CMV).

Picker's team has been developing this vector for years, and has moved from success to success. They have found that after they vaccinated monkeys with their CMV vector, all monkeys got infected after repeated challenge with SIV, the simian version of HIV. But of those monkeys who received the vaccine, half appear to have durably suppressed—and perhaps eliminated—the infection, and done so using novel immunological mechanisms.

The making and possible uses of a trimer

HIV uses a toadstool-like structure on its surface—known variously as the spike or envelope protein—to latch onto and invade T cells. This envelope is, in molecular terms, a heterotrimer. It is made up of three sets of two identical proteins, one known as gp41 that traverses the viral membrane (the toadstool's stem), and another called gp120 (its bulbous top), which binds to a molecule named CD4 on T cells. Even when on the virus, the complex tends to fall apart, leaving clusters of gp41 stems on the surface of HIV that serve as decoys to the antibody response.

This is one major reason that researchers have not been able to study the structure of the envelope, or to use it as an immunogen. Nor have they been able to crystallize the intact, functional protein, a step needed to assess its structure at a practically relevant resolution. The researchers who made the BG505-SOSIP.664 trimer (see main story) sought to work around both these problems.

To do so, they took a gene for the envelope from a founder virus—the one thought to have caused infection—isolated from an infant in Kenya. They then engineered the gene to improve the formation and stability of the trimer and lopped off the part of the stem that crosses the membrane to make sure the resulting proteins wouldn't clump together in water.

Viewed under the electron microscope, it looks from above exactly like the naturally occurring protein. And pretty much every one of the mimics the researchers examined looks this way, attesting to its stability. Closer analysis of its structure using x-ray crystallography and a special kind of electron microscopy confirms this finding.

So do the biochemical studies of the trimer. Not only does the mimic bind broadly neutralizing antibodies (bNAbs) that require an exactly right structure to the trimer, it also fails to bind all those non-neutralizing antibodies that target decoys made by malformed trimers.

All this matters because it suggests that the mimic might be useful as an immunogen to elicit bNAbs. But that will apparently require a little more work. Initial studies in which the mimic was used to elicit immune responses in rabbits resulted in the production of antibodies capable of neutralizing only the virus from which the mimic has been derived. It could not neutralize a random panel of HIV strains that are relatively difficult to block. So the current mimic doesn't quite fit the bill for an HIV vaccine candidate—at least not yet. But studies continue on this front even with the current mimic.

So how can researchers use this mimic, or another like it, to make a bNAb-inducing immunogen? The research team contemplates a number of strategies to that end. Researchers might, for example, trim or chemically cloak elements of the trimer known to be highly variable. These elicit ineffective antibody responses as well, so their removal might focus the immune system on more effective targets.

They are also planning studies in animals now to use the trimer mimic in combination with another immunogen that has been devised to mimic the specific molecular shape targeted by a potent bNAb. Other strategies along these lines would be to use a series of evolving trimer mimics as boosts following a priming vaccination, or a cocktail of similar mimics derived from other HIV viruses. Those would, however, have to be constructed, and there's no guarantee other trimers would be as amenable to useful manipulation as the one obtained from the founder virus that gave us BG505-SOSIP.

If any one of these strategies does elicit anything like a neutralizing antibody response in animal and laboratory studies, it would be considered a significant breakthrough. But it bears mentioning that BG505-SOSIP represents just the first step of a long journey. Any immunogen made on the basis of this mimic would still have to run the gauntlet of preclinical proof and clinical trial that are required for the licensing of a vaccine. —*UK*

Those mechanisms appear to take effect even when non-replicating versions of the vector are used. Picker said work has begun to develop a cytomegalovirus HIV vaccine vector for evaluation in humans.

Ronald Desrosiers of Harvard University's New England Primate Research Center has been working on an analogous strategy for vaccination. He and his colleagues have, however, built their SIV vaccine vector from the rhesus rhadinovirus, which also causes a chronic infection in monkeys. Desrosiers reported that three of five monkeys given this vaccine and subsequently infected with SIV suppressed their SIV infection to almost undetectable levels. His analysis also suggests that antibodies might have played a role in the observed protection.

Most of these vectors, and the immunogens being designed to elicit effective neutralizing antibodies, are in the earliest stages of development. Many—perhaps most—will stumble along the arduous path to licensure. But if any single thing was clear in Barcelona, it was that the odds are improving in favor of at least one of these strategies resulting in an effective HIV vaccine. ■

GLOBAL NEWS *by Andreas von Bubnoff*

Primate studies suggest antibodies could be used to treat HIV infection

Researchers have in the past few years found many new broadly neutralizing antibodies (bNAbs) that are often much more potent and broadly effective against HIV than the few that were available before 2009. One goal now, of course, is to develop a vaccine that can elicit these bNAbs. But researchers are also interested in using them to treat HIV infection. Last year, they reported for the first time that a cocktail of several bNAbs could for some time suppress HIV replication in mice that carry human immune cells.

Now researchers report even more impressive effects of this approach in rhesus macaques chronically infected with SHIV, a monkey variant of HIV that carries the HIV envelope protein on its surface, suggesting that bNAb treatment might even work in HIV-infected humans. In two studies, researchers showed that injecting a cocktail of two or three bNAbs could lower viral loads to undetectable levels within a week. The effect lasted several weeks to several months and was dependent on the continued presence of the infused antibodies in the body.

In one study, led by Dan Barouch of Beth Israel Deaconess Medical Center and the Ragon Institute, administration of just one bNAb called PGT121 kept the virus at undetectable levels for about two months, an effect that was more dramatic than what had been seen in the mouse study last year. "After we did our first experiments, the results were so dramatic that we simply had to do the experiment again a second time to make sure that we all believed the results," Barouch says. "Even the PGT121 antibody [alone] worked in these monkeys, so it was surprisingly effective," observes Louis Picker, who was not connected to the studies but wrote a commentary about them in the same issue of *Nature*, the scientific journal where both studies appeared.

Barouch and colleagues also found that, as expected, the virus resurged once the bNAbs disappeared from the blood of the monkeys. But it reappeared at lower levels. What's more, the three PGT121-treated animals with the lowest initial virus levels suppressed the virus even after antibody levels had become undetectable. This suggests that the bNAb treatment had improved immune function, and indeed, Barouch and colleagues found that the function of $CD4^+$ and $CD8^+$ T cells was improved in the treated animals.

That's not to say that there were no limitations of the treatment: PGT121 didn't fully suppress virus in the animals with the highest initial viral loads. And in the other study, led by Malcolm Martin of the National Institute of Allergy and Infectious Diseases, some animals became resistant to one of the bNAbs used in the experiments. This suggests that researchers may have to combine several bNAbs to make sure resistance is less likely.

The monkey results suggest that the treatment may also work in humans, and Barouch says his group and others are now interested in exploring a number of those antibodies in clinical trials.

Still, Picker says, the question is whether bNAb treatment will add anything to current antiretroviral therapy regimens for routine treatment of HIV infection, given that current antiretroviral drugs can keep virus levels undetectable by just taking a pill a day and can enter the CNS; in contrast, generating the bNAbs of the high quality needed for human use isn't cheap, and the antibodies have to be injected and potentially can't enter the CNS.

But Martin says that bNAbs might be useful in certain situations where one can't use drugs, such as in people resistant to all drugs, or in newborns. What's more, some of the effects of bNAbs go beyond those of antiretrovirals (ARVs). In the monkey studies, bNAbs seemed to suppress virus to undetectable levels faster than do ARVs. That's probably because unlike ARVs, bNAbs can eliminate free HIV from blood.

Also unlike ARVs, bNAbs can mediate the killing of infected cells by other parts of the immune system once they bind to HIV proteins some infected cells display on their surface. One indication that this might be happening is that Barouch and colleagues found that the antibody treatment reduced the number of cells with integrated HIV DNA in blood, lymph nodes and gut lining of the monkeys.

Because bNAbs can kill HIV-infected cells, Picker says they might also be able to lower the residual virus burden in people on highly active antiretroviral therapy, which could help reduce the chronic virus-related inflammation that is thought to contribute to long term complications such as premature aging and accelerated cardiovascular disease.

bNAbs might even help with strategies researchers are developing to cure HIV infection, where the challenge is to get the virus out of its hiding place in latently infected, resting memory $CD4^+$ T cells. These cells harbor integrated HIV DNA in their genome, and one strategy to eradicate this "HIV reservoir" is to activate the latently infected cells so that they produce virus again. These reactivated cells can then be targeted for elimination, and the new studies suggest that bNAbs might be a good way to kill them. "I believe that these antibodies have a definite role in the next generation of strategies that will be evaluated to try to reduce and hopefully eventually eliminate the viral reservoir," Barouch says. ■